

Ultramorphology and Histology of the Foregut and Midgut of *Pachycondyla* (= *Neoponera*) *villosa* (Fabricius) Larvae (Formicidae: Ponerinae)

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Abstract.—We studied the foregut and midgut of larvae of *Pachycondyla* (= *Neoponera*) *villosa* (F.) using histological methods and the scanning electronic microscope. In this paper we discuss the muscle layers of these regions, the origin of peritrophic matrix, the digestive cells secretion process, and the term proventriculus used in larvae.

The digestive tract of hymenopteran larvae consists of the pharynx, esophagus, proventriculus, ventriculus and ileum (Wheeler 1926, Nelson 1924, Wheeler and Wheeler 1976). When compared to the adult digestive tract, the major difference is that the foregut of the larva has no crop and in the hindgut there is no clear difference between ileum and rectum. The larval hindgut consists of a short and narrow tube which widens at the end. The Malpighi tubules open into the anterior portion of the ileum.

The ventriculus occupies most of the larval body and represents the major portion of the gut both in terms of length and diameter. The peritrophic matrix, which is well developed in larvae, is practically absent in adults, where it is found in few cases (Caetano 1988, Caetano et al. 1986/1987, Caetano and Hoffmeister 1987, Caetano et al. 1994).

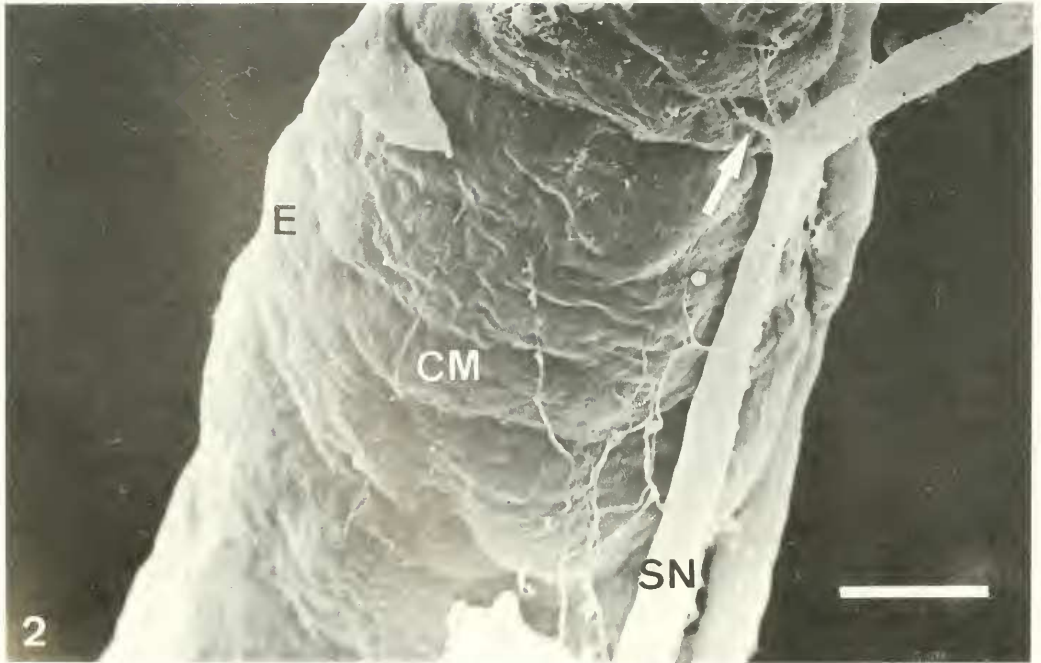
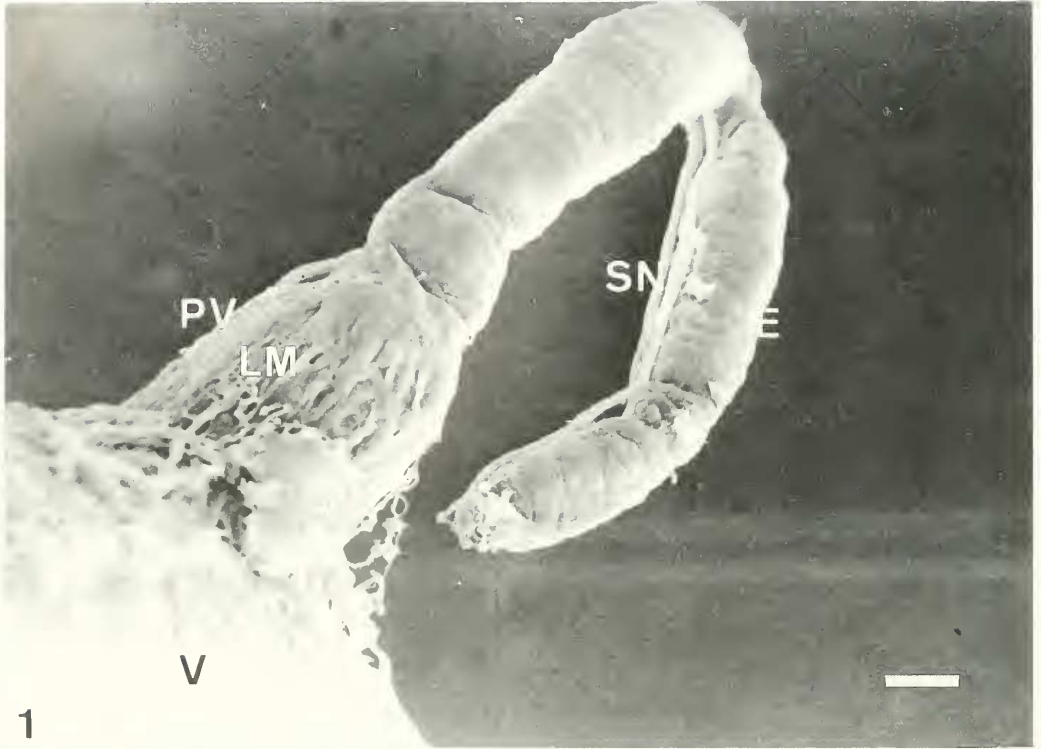
The major objective of the present study is to describe some ultramorphological and histological aspects of larvae foregut and midgut of the *Pachycondyla villosa* (F.)

and to present some ontogenetic considerations.

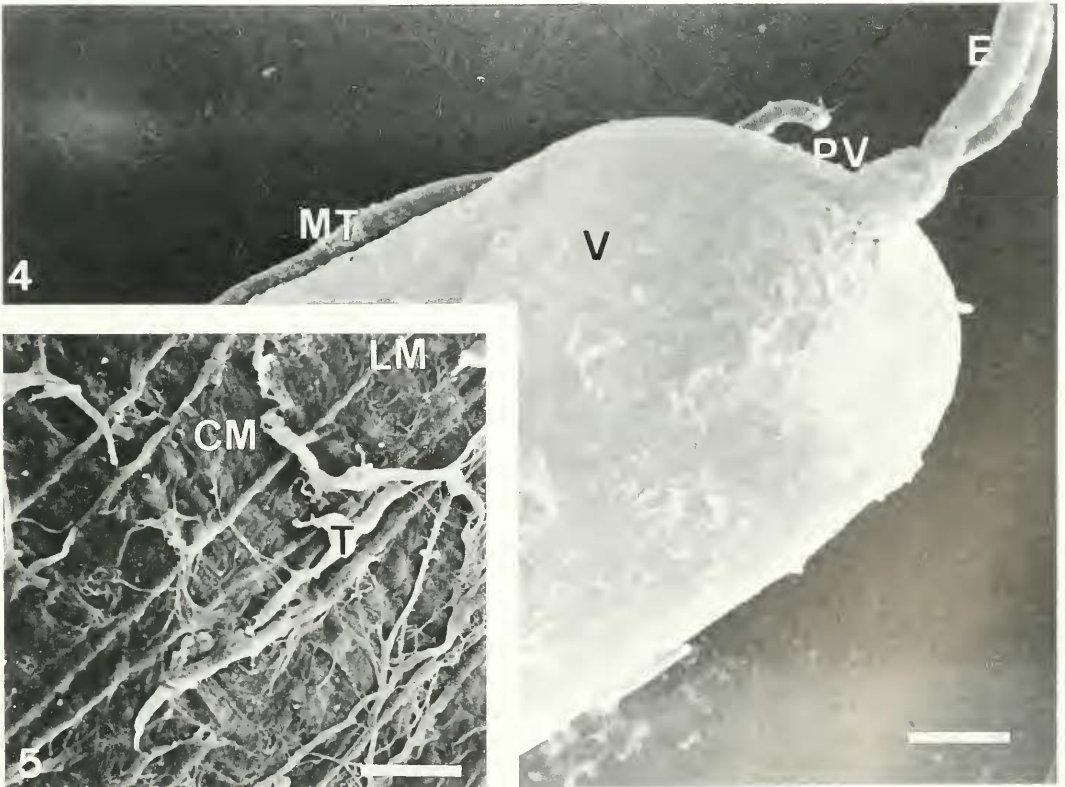
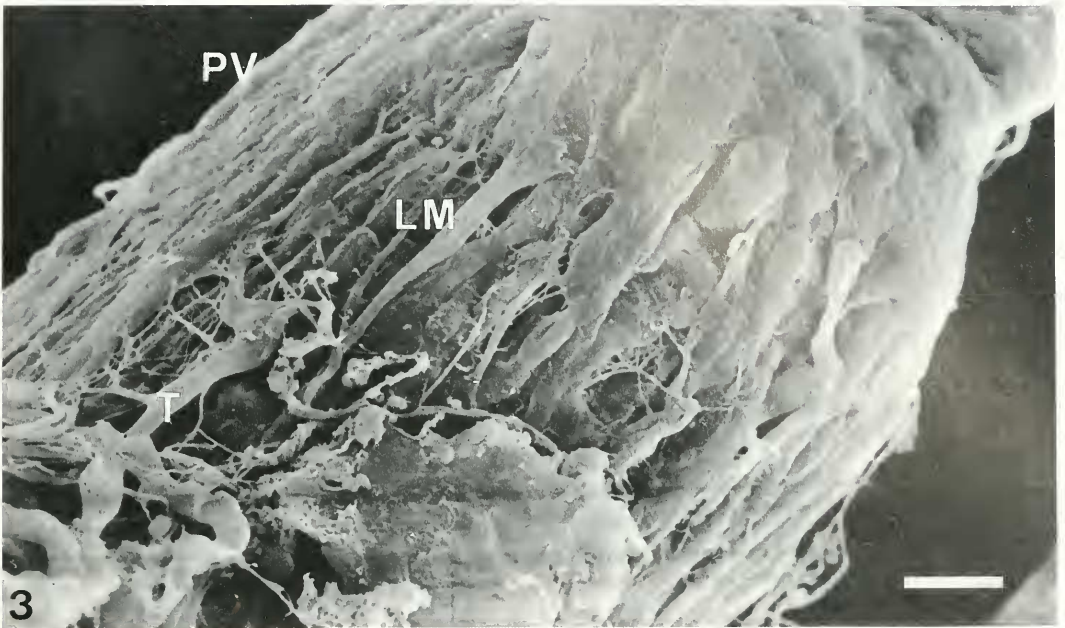
MATERIALS AND METHODS

For ultramorphology study, *P. villosa* larvae were dissected in physiological saline for insects in a Petri dish covered with colored wax. The digestive tract was removed and fixed in Karnovsky fluid for 24 hours, dehydrated in an ascending alcohol series (70 to 100%), subjected to two acetone 100% baths of 15 minutes each and then critical pointed dried (Balzers CPD 030). After dehydration the material was placed on aluminum supports attached with double-faced tape and sputtercoated with gold (in sputtering Balzers SD 050). The digestive tract was examined with a Jeol P15 SEM and photographed on Neopan SS 120 film.

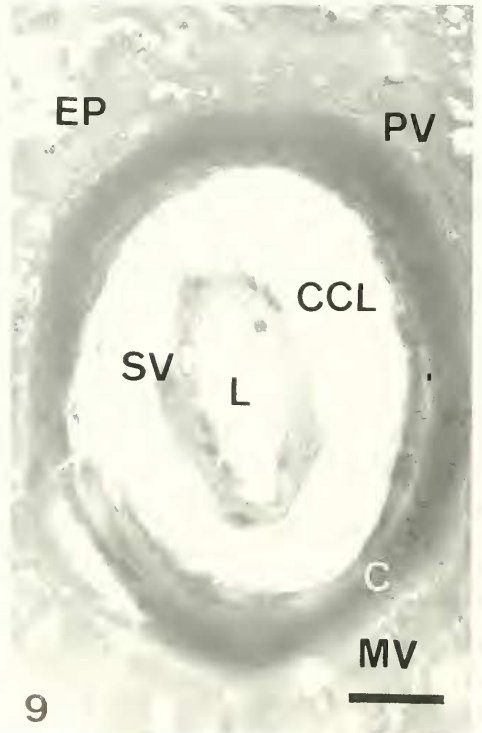
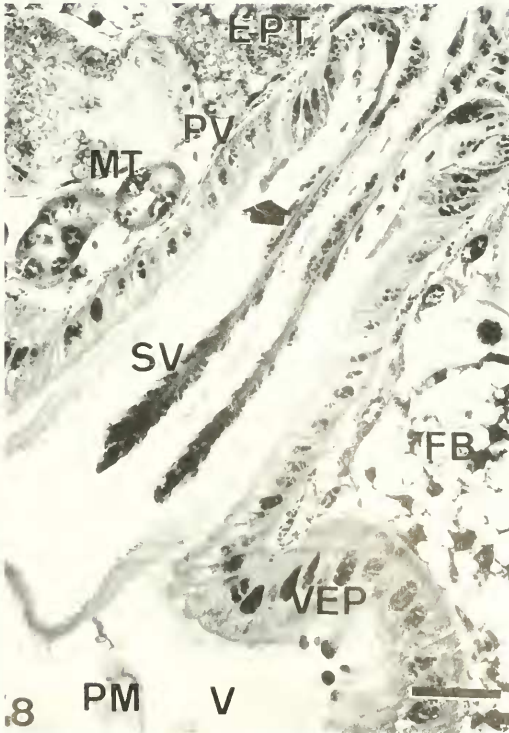
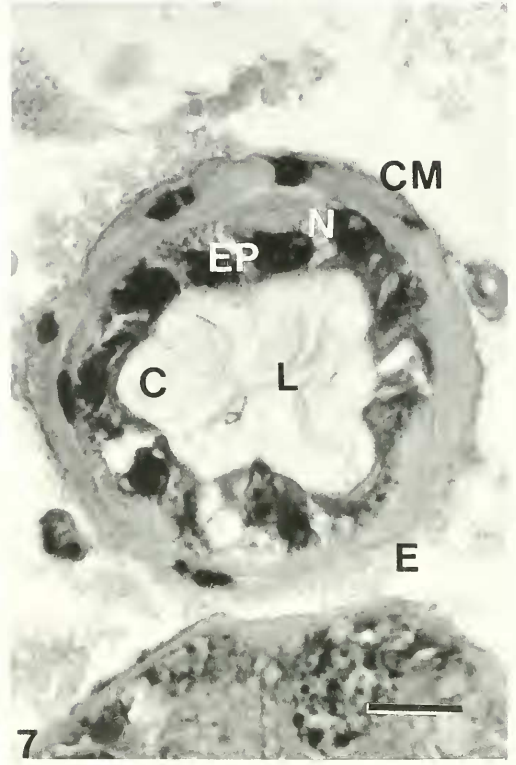
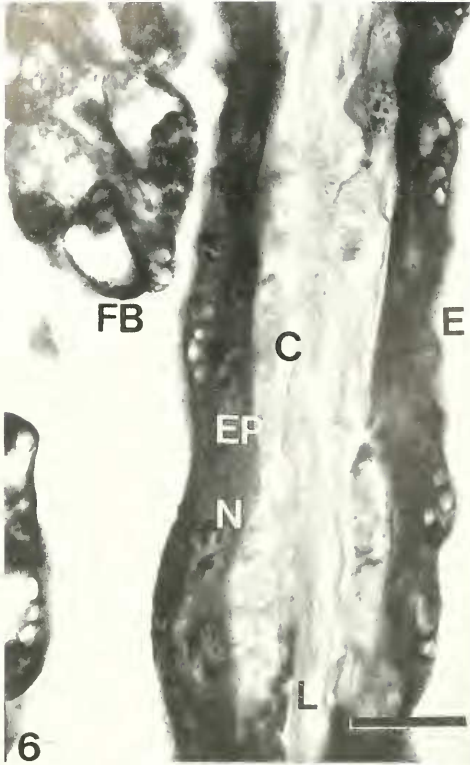
For historesin preparation, *P. villosa* larvae were fixed directly in 4% paraformaldehyde in phosphate buffer (0.1 M, pH 7.4). The material was then dehydrated in 70, 80, 90 and 95% ethanol solutions for 20 minutes and transferred to resin solution (JB4—Polaron Instruments/Bio Rad) for



Figs. 1-2. 1, General view of foregut. E = esophagus; SN = stomogastric nerve; LM = longitudinal muscle; PV = proventriculus; V = ventriculus. Scale bar = 40 μ m. 2, Detail of esophagus showing relationships with stomogastric nerve (arrow). CM = circular muscle. Scale bar = 10 μ m.



Figs. 3-5. 3, Proventriculus detail where longitudinal muscle occurs externally. Scale bar = 20 μ m. 4, General view of mesenteron (V = ventriculus). MT = Malpighian tubules. Scale bar = 200 μ m. 5, Detail of ventriculus wall showing thin and external longitudinal muscle and under these fibers the stronger circular muscle. T = trachea. Scale bar = 30 μ m.



72 hours at 4°C. Finally the material was transferred to molds filled with resin containing a catalyze and sealed with metal support for microtomy. The blocks were cut in Sorvall JB4/Bio Rad microtome. The sections were stained with hematoxylin-eosin and photographed with Zeiss photomicroscope.

RESULTS AND DISCUSSION

Ultramorphology.—The esophagus is a short and narrow tube that dilates near the ventriculus to form the proventriculus (Fig. 1). This region is covered with a circular muscle layer (Fig. 2) that does not resemble that observed in the adults of other ants (Caetano 1990). In the adults, the external muscle is visibly striated and consists of oblique fibers that intercross, bifurcate or anastomose. In the larvae there were no such striations or fiber separation. The image is that of a sheath covering the entire organ. The circular muscle of the esophagus stop abruptly at the anterior border of proventriculus and resemble those described to *Soleuopsis invicta* Buren (Petralia and Vinson 1980) and other ant larvae (Valentini 1951).

The stomogastric nerve runs along the entire larval esophagus towards which it emits small branches that penetrate the muscle sheath (Figs. 1 and 2), as is also observed in adults.

The proventriculus appears as a protrusion from the ventriculus surface (Fig. 1). This protrusion resembles that described by Caetano (1988), Caetano et al. (1986/

1987) and Tomotake (1990) for adults of the subfamily Ponerinae and called button. Eisner (1957) and recently Tomotake et al. (1995a,b) have shown that in adults the proventriculus may have external longitudinal muscle fibers, as also observed for some species of the subfamily Ponerinae (Tomotake 1996). The presence of these longitudinal fibers in adults suggests that they may have retained this character from larvae because the proventriculus is the only foregut region that has superficial longitudinal muscle fibers in both adults and larvae. Structurally this arrangement does not differ from typical figures of larvae presented in morphology textbooks (Wheeler and Wheeler 1976). The most clearly visible elements are the outer longitudinal muscle fibers (Fig. 3), which were also observed in this portion of the digestive tract of *P. villosa* adults by transmission electron microscopy Caetano (1991).

The ventriculus is elliptical, very wide (Fig. 4) and covered with weakly developed longitudinal muscles and connective tissue. The fibers of the circular muscle are located below these layers (Fig. 5). The location of these muscles follows the pattern known for adults of these and other ant species in which a web of amorphous connective tissue is observed (Caetano unpublished data) through which tracheal branches penetrate the ventriculus (Fig. 5).

According to Lappano (1958), Petralia and Vinson (1980) the ventriculus is the largest organ of the larva and appears to

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Figs. 6-9. 6, Longitudinal section of esophagus, showing thick cuticle (C) covering lumen. FB = fat body; L = lumen; N = nucleus; EP = esophagus epithelium. Scale bar = 10µm. 7, Transverse section in esophagus showing thick cuticle, cubic epithelium of esophagus and sheath of circular muscle. Scale bar = 20µm. 8, Section through proventriculus showing different epithelia of region. (EPT) transition epithelium, (SV) stomodeal valve epithelium and (VEP) ventricular epithelium. In this micrograph we can observe that the transition epithelium is producing the peritrophic matrix (arrow). The Malpighian tubules and the fat body are close to this region. PM = peritrophic matrix. Scale bar = 20µm. 9, Transverse section in proventriculus. MV = microvilli; SV = stomodeal valve; L = stomodeal valve lumen; C = cuticle; CCL = stomodeal chamber lumen. Scale bar = 20µm.

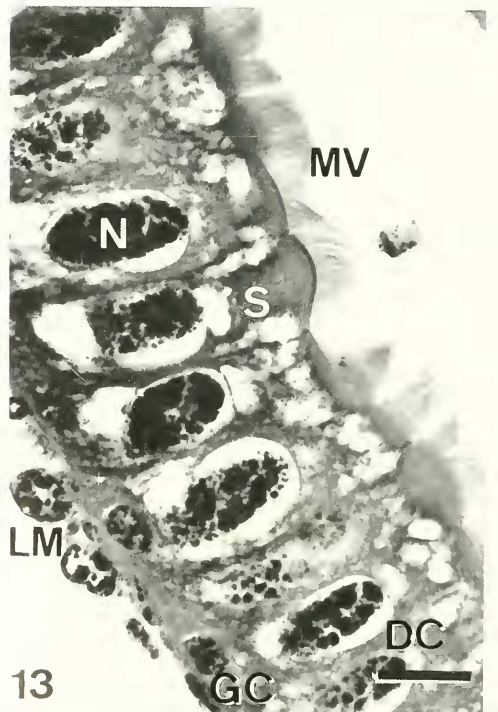
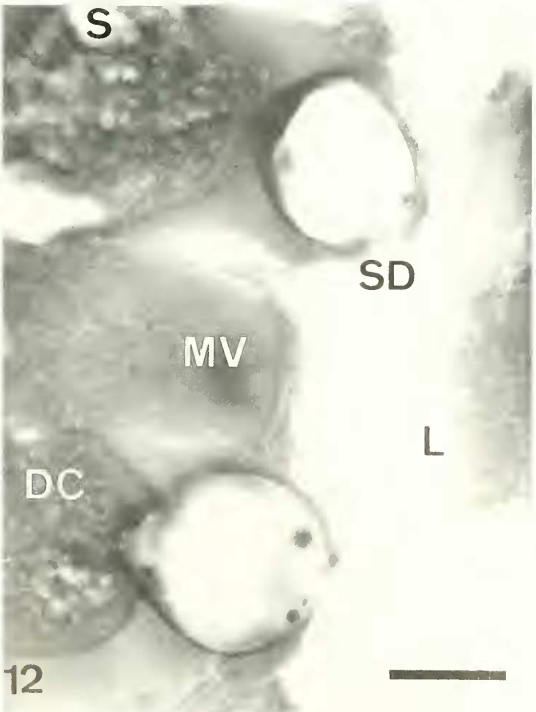
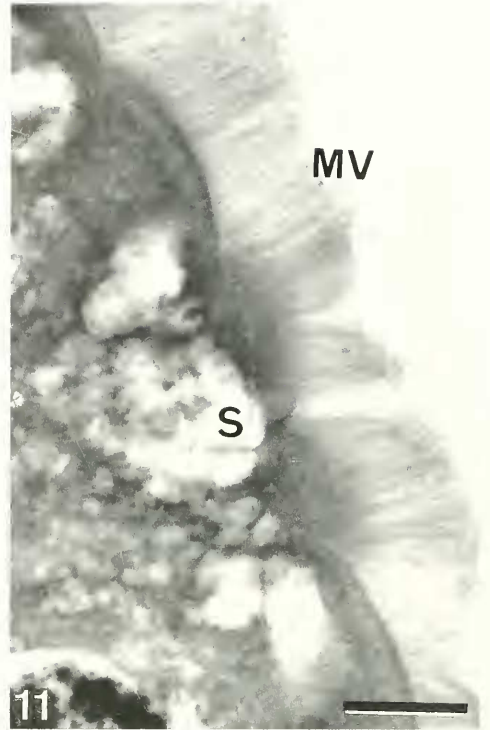
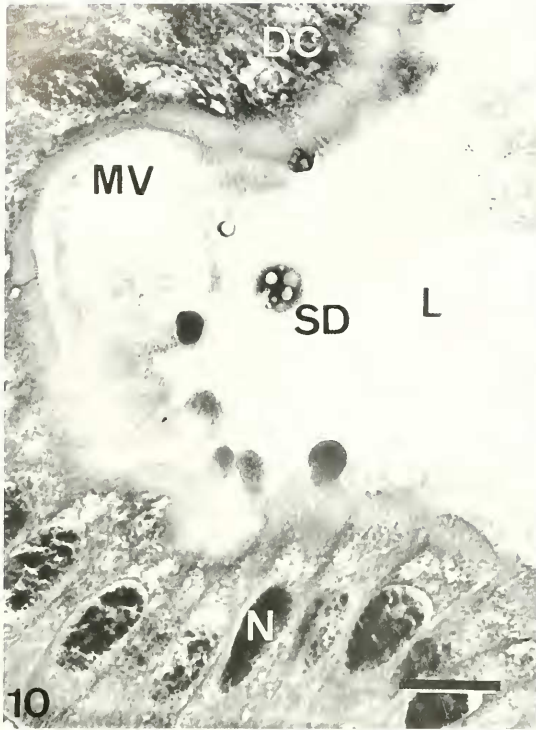


Fig. 10-13. 10, Ventriculus epithelium with digestive cells (DC); cells have large median nuclei (N) and long microvilli (MV) with smooth secretion droplets (SD) between them. Scale bar = 20µm. 11, Detail of apex of

serve as a mould around which the other abdominal organs are orientated. This suggestion is confirmed in *P. villosa* larvae.

Histology.—The esophagus has a wide lumen, cuboidal epithelium covered with a thick cuticle and a circular muscle sheath covering it externally (Figs. 6 and 7). During feeding, the food bolus may be transported through the esophagus by means of peristalsis, as occurs in *S. invicta* (Petralia and Vinson 1980). Except for the wide lumen and thick cuticle, this description agrees from that of adults of the same species (Caetano 1988) and of all other adult ants studied so far (Walker and Clower 1961, Caetano and Lage Filho 1982, Caetano 1984, 1988, 1990). In adults the epithelium is thin and the muscle sheath less so evident.

The proventriculus consists of columnar epithelium with basal nuclei and well developed ensheathing longitudinal muscles. This portion of the foregut has two chambers: one formed by the epithelial portion of the foregut (cardiac or stomodeal valve), and the other located between the stomodeal valve and the proventriculus wall, whose lumen communicates directly with the lumen of the ventriculus (Fig. 8).

Thus, the proventriculus of *P. villosa* larvae appears to be formed by a prolongation of the epithelium of the ventriculus towards the foregut and its internal portion, the stomodeal valve proper, is formed by a projection of the foregut towards the ventriculus (Fig. 8). Thus, these portion probably reflect a mixed developmental origin: the internal part arises from the ectoderm of the foregut and the outer part from the endoderm of the mid-

gut. The presence of outer longitudinal muscle fibers in the so-called larval proventriculus, as observed in the ventriculus, is clarified. On this basis, we believe that in larvae the structure known as proventriculus should be called "stomodeal chamber" because of its position and it harbors the stomodeal valve, in contrast with the adult proventriculus that does not have the stomodeal chamber (Caetano 1988).

The lumen of this region is lined with a thick cuticle organized in a trabecular formation, which does not resemble the cuticular arrangement of adults. The adult proventricular lining is organized into four (or more) mobile lips that are usually covered with spiniform cuticular structures (Eisner 1957, Caetano et al. 1991, 1998, Tomotake 1996).

The ectodermal origin of the stomodeal valve was indicated by the presence of cuticle that does not stain in histochemical processes for proteins, as shown in transverse sections (Fig. 9).

The ventriculus is a yellow or dark coloured region visible in live larvae. It is a blind sac posteriorly, similar to what is observed in several species of higher Hymenoptera as *Apis mellifera* L. (Nelson 1924), *Eciton burchelli* Westw. (Lappano 1958), *S. invicta* (Petralia and Vinson 1980), *Ectatomma edentatum* Roger (Zara and Caetano 1998) and some species of the subfamily Formicinae, Dolichoderinae and Myrmicinae (Valentini 1951), although Athias-Henriot (1947) reports it to be open in *Monomorium*.

The ventricular epithelium is columnar and could be identified by one type of digestive cell. This cell has distinct round or oval pycnotic nucleus located

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digestive cells with long microvilli and secretion (S) close to apical membrane. Scale bar = 10µm. 12, Detail of secretion droplets (SD) releasing from digestive cells. Note absence of microvilli in secretion droplet. Scale bar = 10µm. 13, General view of ventriculus epithelium of old larvae showing large space around nucleus and secretion in apex. GC = Regenerative cell. Scale bar = 20µm.

between the median and basal portion of the cell (Fig. 10) similar to *E. burchelli* and *S. invicta* (Lappano 1958, Petralia and Vinson 1980, respectively). The apex of these cells is rounded, conferring a club-like shape on them, and usually appears to contain large secretion vacuoles. The border bears long microvilli (Figs. 10 and 11). The secretion is released, like a "budding" process in the form of vesicles containing granules that stain differentially with H-E (Figs. 10, 11 and 12). This process resembles that observed in the adult ventriculus of *Pachycondyla striata* Smith, but without microvilli surrounding the vesicles (Caetano et al. 1994). This kind of release secretion is different than merocrine secretion described in *Cataglyphis bombycina* (Roger) larvae (Valentini 1951). These cells could be producing digestive enzymes; however, the absorptive cells were not observed.

The nests of regenerative cells commonly present in the ventriculus of adults are not observed in larvae (Caetano 1984, 1988, 1990, Caetano et al. 1986/1987), but they occur as isolated nuclei close to the basal lamina (Fig. 13).

The peritrophic matrix is clearly visible in ventricular lumen (Figs. 14 and 15) and an accumulation of "membranes" is noted in its distal portion. Some of the membranes enclose food remains and some of them are empty (Fig. 16). In this region, the remnants of the peritrophic membrane (meconium) are accumulated for later elimination during pupation as also observed in *S. invicta* (Petralia and Vinson 1980). The anterior region of the proventriculus, more precisely the transition epithelium (Fig. 8, arrow), seems to be responsible for the secretion of the peritrophic matrix which expands soon after leaving the cardiac valve (Fig. 14) and reaches the epithelium of the ventriculus. This kind of peritrophic matrix formation is similar to that recorded for *E. edentatum* larvae (Zara and Caetano

1998). In contrast, Petralia and Vinson (1980) described a second type of peritrophic matrix produced along the entire ventriculus of *S. invicta*, but this does not occur in *P. villosa*.

In adults of some ponerine genera (e.g., *Ectatomma* and *Neoponera*), Caetano (1988) reported a similar origin of the peritrophic matrix, which at first may be confused with the stomodeal valve itself. In the adults, however, the peritrophic matrix penetrates the median region of the ventriculus and opens, appearing to originate from inside the stomodeal valve. In the larvae studied here the peritrophic matrix opens immediately upon leaving the stomodeal valve, which serves as a mold.

CONCLUSIONS

a) The outer muscles present in the foregut and midgut of *P. villosa* larvae differ from those of adults by the absence of clearly transverse striations.

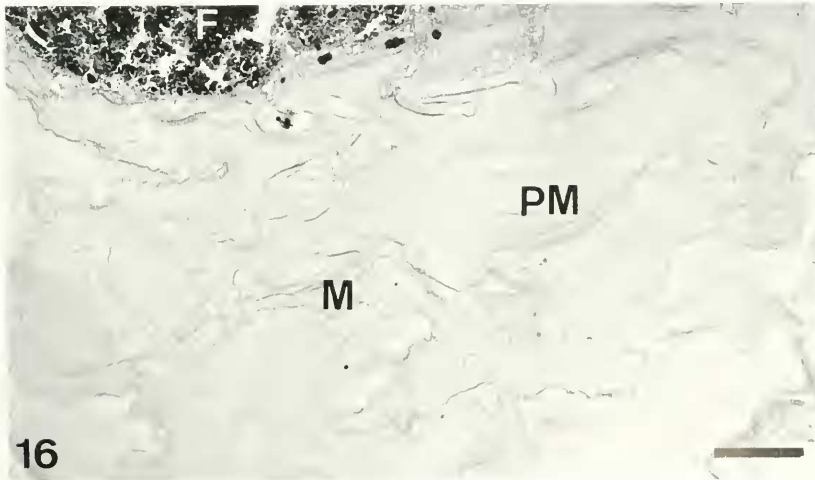
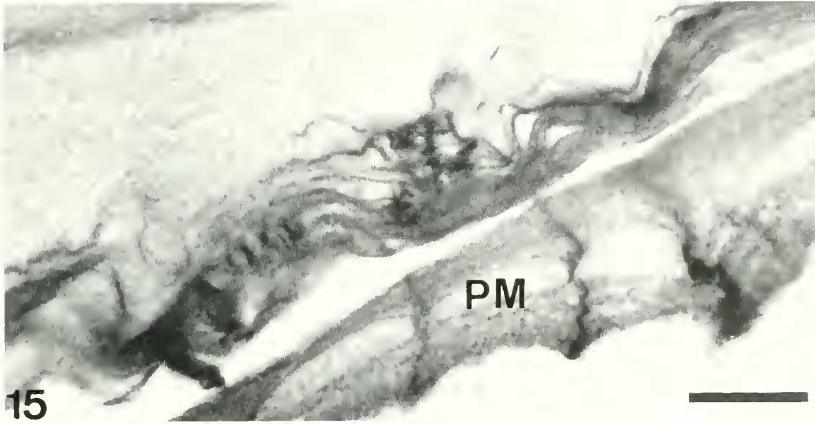
b) The proventriculus and the ventriculus of the larvae have an external longitudinal muscle sheath but only the adults ventriculus shows it.

c) The larval proventriculus is morphologically simpler than in adults; the presence of cuboidal epithelium with microvilli indicates a secretory function.

d) The proventriculus term used for adults does not seem to be appropriate for the larval structure described here, we suggest the stomodeal chamber.

e) The "button" located close to the ventriculus anterior region previously described by other authors and present in adults of the subfamily Ponerinae is similar to the upper portion of the stomodeal chamber.

f) The histological characteristics of the stomodeal chamber of larvae lead us to believe that this portion may originate from the endoderm as the ventriculus.



Figs. 14-16. 14, General view of ventriculus proximal region with stomodeal valve and peritrophic matrix being released. Scale bar = 10 μ m. 15, Close up of peritrophic matrix showing its layers. Scale bar = 10 μ m. 16, General view of posterior region of ventriculus with "meconium" (M). Scale bar = 50 μ m.

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